OUTLINE OF THE PROPOSED WORK FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES

Submitted To

SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY, NANDED.

Topic

"DEVELOPMENT AND EVALUATION OF POLYHERBAL FORMULATION FOR THE MANAGEMENT OF CHRONIC DISORDER"

Submitted by

Mr. Shivakumar S. Ladde

M. Pharm. (Pharmacology)

Supervised By

Dr. Bhusnure O. G. **M. Pharm, Ph. D.**

Principal

Channabasweshwar Pharmacy College (Degree), Latur, Maharashtra, India - 413512

स्वामी रामानंद तीर्थ
मराठवाडा विद्यापीठ,नांदेड.

(2017-2018)

Swami Ramanand Teerth Marathwada University,

Nanded-431606, (Maharashtra State), India

Swami Ramanand Teerth Marathwada University,

Nanded-431606, (Maharashtra State), India.

PROFORMA FOR REGISTRATION OF SUBJECTS FOR DISSERTATION

1	**Name of the candidate and Address**	**SHIVAKUMAR, S/o. Sanmukappa Ladde, Teen Dukhan Galli,Bhalki-585328, District: Bidar, Karnataka.**
2	**Name of the Institution:**	**Shivlingeshwar College of Pharmacy, Almala- 413520, Tq-Ausa, Dist-Latur, Maharashtra**
3	**Course of study and Subject:**	**Doctor of Philosophy in Pharmaceutical Sciences**
4	**Date of admission to the Course:**	
5	**Title of the topic:**	
6	**Brief resume of the intended work:** **6.1: Introduction** **ENCLOSURE –I** **6.2: Review of Literature** **ENCLOSURE -II** **6.3: Need for the study** **ENCLOSURE -III** **6.3: Main objective of the study** **ENCLOSURE -IV**	
7	**Material and methods:** **7.1: Source of the data** **ENCLOSURE -V** **7.2: Methods of the collection of the data** **ENCLOSURE -VI** **7.3: Does the study require any investigations or interventions to be conducted on patients other human or animals? If so, please describe briefly. YES (mice and rats)** **7.4: Has ethical clearance been obtained from your institute in case of 7.3** **YES: Reg.no.**	
8	**List of References**	**ENCLOUSUR-V**
9	**Signature of the candidate:**	**(SHIVAKUMAR S. LADDE)**
10	**Remarks of the guide:**	
	Name and designation of **11.1 Guide:** **11.2 Signature**	**Dr. Bhusnure O. G.** **M. Pharm, Ph. D.** **Principal** Channabasweshwar Pharmacy College (Degree), Latur, Maharashtra, India - 413512

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ENCLOSURE –I

6. Brief resume of the intended work:

6.1 INTRODUCTION:

Quality by Design (QbD) refers to a holistic approach towards drug development. Quality by design is a vital part of the modern approach to pharmaceutical quality. QbD was first described by Joseph M. Juran, and applied heavily, particularly in the automotive industry. A summary of the drug development program described in terms of labeling concepts and it mainly focus on the safety and efficacy[1]. The primary focus of pre-clinical drug development is to evaluate the safety of the drug product through in vitro assays and animal studies. In vitro assay and animal testing are often considered as surrogate for human testing, under the assumption that they can be predictive of results in humans. Basically, pre-clinical drug development includes the stages of chemical synthesis, screening for activities, and pre-clinical testing. The purpose of drug screening is to identify a stable and reproducible compound with fewer false-negative and false-positive results. For this purpose, a multiple-stage screening procedure (design) is usually employed. Pre-clinical testing involves dose selection, toxicological testing for toxicity and carcinogenicity, and animal pharmacokinetics.

In recent years, the concept of quality by design in (global) pharmaceutical development has received much attention. The purpose is to ensure that the compound under investigation will possess good drug characteristics such as identity, strength, purity, quality, safety, efficacy and stability before and post approval. A pharmaceutical development process consists of non-clinical (e.g., assay/process validation and stability testing), pre-clinical (e.g., animal and bioavailability/bioequivalence studies), and clinical (e.g., phases 1-3 clinical trials) development[2]. The primary objective of the pharmaceutical industry is to create new therapies to solve unmet medical needs to advance the human condition. To date, this pursuit has involved the use of numerous animals of various species in a wide variety of models and testing paradigms. There are a number of recent trends in the pharmaceutical industry environment that impact preclinical model usage and practices. One of the most impactful has been the significant increase in the globalization and externalization of discovery activities.

The cornerstone of international policy on animal-based science is embodied in the replacement, reduction, and refinement (3Rs) set out in the now famous treatise of R. Russell and W. M. S. Burch entitled ‘‘The Principles of Humane Experimental Technique’’. Replacement refers to the preferred use of non-animal methods over animal methods

whenever it is possible to achieve the same scientific aim. Reduction refers to methods that enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals. Refinement is much broader and refers to methods that alleviate or minimize potential pain, suffering, or distress, and enhance animal welfare for the animals still used. Preclinical animal studies to be conducted much more akin to the methodology of randomized clinical trials using similar methods for bias control (randomization, blinding, sample size justification, data transparency, etc.

The concept of the 3Rs of relevance, robustness, and reproducibility is put forth as a transformational cultural change for bioscience research personnel to embrace to enhance the translational value of preclinical animal efficacy studies. The present inability to show good concordance between some animal efficacy studies and human clinical outcomes is believed to be due in part to short comings in experimental design and conduct, as well as in the reporting of results. It is believed that the reproducibility and value of preclinical animal efficacy studies would be enhanced by increased methodological rigor in both study conduct and reporting. An increased emphasis on protocol development with a focus on sources of variation and methods to control bias would enhance preclinical animal study quality[3].

A chronic condition or illness, in general terms, is a condition that is slow in progression, long in duration, and void of spontaneous resolution and it often limits the function, productivity, and quality of life. Chronic diseases generally cannot be prevented by vaccines or cured by medication, nor do they just disappear. Eighty-eight percent of Americans over 65 years of age have at least one chronic health condition. Health damages behaviors - particularly tobacco use, lack of physical activity, and poor eating habits - are major contributors to the leading chronic diseases. Chronic diseases tend to become more common with age. Chronic diseases, such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes, are by far the leading cause of mortality in the world, representing 60% of all deaths. Out of the 35 million people who died from chronic disease in 2005, half were under 70 and half were women[4]. The leading chronic diseases in developed countries include arthritis, cardiovascular disease such as heart attacks and stroke, cancer such as breast and colon cancer, diabetes, epilepsy and seizures, obesity, and oral health problems.

Oxidative stress causes an imbalance between the generation of reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation in vivo and in vitro and create oxidative stress which results in numerous diseases and such as cancer,

cardiovascular disease, neural disorders, Alzheimer's disease mild congnitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, ageing, atherosclerosis through are cytotoxic effect and tissue injuries. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury. Molecules such as vitamin C and E inhibit lipid peroxidation in cell. When the mechanism of antioxidant protection becomes unbalanced in human body, antioxidant supplement may be used to help reduce oxidative damage[5].

ENCLOSURE-II

6.2 REVIEW OF LITERATURE

Han J. et al (2008) studies evaluates the effect of exogenous administration of melatonin on the expression of morphine reward in mice using a conditioned place preference (CPP) paradigm. The conditioned place preference was induced by morphine (s.c., 3 mg/kg, once each day for 5 consecutive days) in mice. Our data showed that the intraperitoneal (i.p.) administration of melatonin (12.550 mg/kg) reversed the expression of morphine induced conditioned place preference in a dose dependent manner. Furthermore, the intracerebro ventricular (i.c.v.) administration of melatonin (0.1250.5 mg/kg) also resulted in dose dependent reversal effect on the expression of morphine induced conditioned place preference. Further investigated which of melatonin receptor subtypes within the central nervous system was mediating this reversal action in mice using luzindole (2benzylNacetyltryptamine, a nonselective antagonist for melatonin MT1 and MT2 receptors) and K185 (N-butanoyl2(5,6,7trihydro11methoxybenzo[3,4] cyclohept [2,1alpha] indol13yl) ethanamine, a selective antagonist for melatonin MT2 receptor). It was shown that the i.c.v. administration of either K185 (5, 20 microg) or luzindole (6.25, 12.5 microg) significantly antagonized the reversal effect of melatonin (50 mg/kg, i.p) on the expression of morphine induced conditioned place preference, while the i.c.v. administration of 20 microg of K185 or 12.5 microg of luzindole by itself did not alter the expression of morphine induced conditioned place preference. These results suggest that melatonin reverses the expression of morphine induced rewarding effect, and this action is mediated by the activation of melatonin MT2 receptor subtype within the central nervous system.[6]

Tajes Orduna M et al, (2009) studies examined the neuroprotective effects of melatonin in an experimental model of aging induced apoptosis. Cerebellar granule neurons show characteristics of apoptosis after 17 days in culture (DV). The addition of melatonin to

neuronal cell cultures (100-500 mum) resulted in neuroprotective and antiapoptotic effects, which were revealed by nuclear condensed cell counting. In a thorough analysis by Western-blot of the potential pathways responsible for melatonin's neuroprotective effects, In study it found an increase in the activation of prosurvival Akt. Subsequently GSK3beta inhibition and an increase in pFOXO1 phosphorylation occurred. In this model of aging, apoptosis was associated with an elevated DNA damage, as demonstrated by an increase in the activation of ataxia telangiectasia muted (ATM). Subsequently, downstream targets such as p53 were activated. Furthermore, the process of DNA damage was coupled to an increase in the expression of certain proteins involved in cell cycle regulation; These were cyclin D and the proapoptotic transcription factor E2F1. It concludes that the antiapoptotic effects of melatonin were mediated by two potential mechanisms: by increasing the activity of prosurvival pathways via Akt and by the prevention of DNA damage (via ATM inhibition) followed by the reduction of cell cycle reentry.[7]

Romina Mancinelli et al, (2011) Studies Normal and BDL rats were treated in vivo with melatonin for 7 days before evaluating: (i) intrahepatic bile duct mass (IBDM) in liver sections by immunohistochemistry for citokeratin-19 (CK-19), a specific marker of cholangiocytes; and (ii) expression of MT1, MT2 and CLOCK genes by immunohistochemistry and real time PCR. In vitro, large cholangiocytes were stimulated with melatonin in the absence/presence of luzindole (a MT1/MT2 antagonist) and 4-P-PDOT (a specific MT2 antagonist) before assessing cellular growth by MTS proliferation assay, cAMP levels by a RIA kit and ERK1/2 phosphorylation by western blots. Cholangiocytes express MT1, MT2 and CLOCK genes that were all upregulated following BDL. Administration of melatonin to BDL rats decreased IBDM and the expression of all CLOCK genes. In cells, melatonin decreased the proliferation, cAMP levels and the activation of ERK2, decreases that were blocked by luzindole. Supporting other studies we found that melatonin-inhibition of biliary hyperplasia is associated with down regulation of CLOCK gene expression. These findings have important clinical implications since melatonin may be an important therapeutic tool for managing cholangiocyte hyperproliferation in biliary disorders.[8]

Claudia Rossini et al, (2011) studies ApoE-deficient mice, (a well-known senescence model) from 6^{th} week to 15^{th} week of life, treated with a specific melatonin formulation: Armonia Retard (kindly provided by Nathura s.r.l, Reggio Emilia, Italy), with an extended-release pharmacokinetic, at different progressive doses 0.04, 0.1, 10 mg/kg/day. We used the same treatment in C57BL6 mice, as control group. Vascular alterations were evaluated in aorta by morphology and immunofluorescence analysis was focused on pleiotropic inflammatory markers, such as interleukins (IL) 6 and 10, inducible nitric oxide synthase

(iNOS), tumor necrosis factor-alpha (TNF-α). We observed in ApoE-deficient mice endothelial cell detachment and IL-6, IL-10, iNOS and TNF-α overexpression. Melatonin treatment improved not only the endothelial damage, but also the overall vascular cytoarchitecture and reduced inflammation and macrophages infiltration. In particular, melatonin Retard at the highest dose, recovered all the above markers to the levels of C57BL6 mice. The results outline the anti-inflammatory and anti-oxidant properties of melatonin and its beneficial anti-aging and anti-atherosclerotic effects, especially in extended-release formulation.[9]

Nutjaree Pratheepawanit Johns et al, (2013) studies assess the melatonin content of six tropical fruits and examined whether human consumption could contribute to dietary melatonin as measured by 6-sulfatoxymelatonin (aMT6s, a marker of circulating melatonin in the body). Melatonin was extracted using methanol and analyzed by high performance liquid chromatography. In a clinical crossover study, 30 healthy volunteers consumed selected fruits one at a time, with a 1week washout period between fruits, until completing all six fruits. Most fruits had moderate melatonin content. Significant increases in urine aMT6s concentrations were seen after the consumption of pineapple (266%, $p = 0.004$), banana (180%, $p = 0.001$), and orange (47%, $p = 0.007$). The need to analyze melatonin both in fruit and as in vivo uptake was demonstrated. Further study is warranted regarding the clinical effect of fruit consumption in people with age related melatonin reduction problems such as sleeplessness and illnesses involving oxidative damage.[10]

Renata Schenkel Rivera Kaminski et al, (2015) studies evaluate the effect of the antioxidants melatonin and N-acetylcysteine on glucose, triglyceride, and cholesterol levels in animals exposed to intermittent hypoxia. In this study Two groups of Balb/c mice were exposed to intermittent hypoxia (n = 36) or sham intermittent hypoxia (n = 36) for 35 days. The intermittent hypoxia group underwent a total of 480 cycles of 30 seconds reducing the inspired oxygen fraction from 21% to 7 ± 1% followed by 30 seconds of normoxia, during 8 hours daily. Melatonin or N-acetylcysteine were injected intraperitonially daily from day 21 on. At day 35, glucose levels were significantly higher in the intermittent hypoxia group than in the control group. The intermittent hypoxia groups receiving N-acetylcysteine and vehicle showed higher glucose levels than the group receiving melatonin. The lipid profile was not affected by intermittent hypoxia or antioxidant administration. From the results it suggests that melatonin prevents the well-recognized increase in glucose levels that usually follows exposure to intermittent hypoxia. Further exploration of the role of melatonin in sleep apnea is warranted.[11]

Beatriz Fernandez-Gil et al, (2017) studies investigate the changes induced in the rat small intestine after external irradiation of the tongue, and to explore the potential radio-protective effects of melatonin gel in Male Wistar rats. Oral treatment with melatonin gel had a protective effect in the small intestine, which was associated with mitochondrial protection and, consequently, with a reduced inflammatory response, blunting the NF-κB/NLRP3 inflammasome signaling activation. Thus, rats treated with melatonin gel showed reduced intestinal apoptosis, relieving mucosal dysfunction and facilitating intestinal mucosa recovery. Their findings suggest that oral treatment with melatonin gel may be a potential preventive therapy for radiotherapy-induced gut toxicity in cancer patients.[12]

Rajput Rekha et al., (2013) studies shows the polyherbal anti-diabetic formulation is prepared in suspension form by mixing the petroleum ether and chloroform extracts of five medicinal plants. In study It was found that the anti-diabetic activity of formulation in suspension form at a dose of 400mg/kg of body weight (253.33± 0.71) more effective than 200mg/kg of body weight (283.33 ±0.49) on Wistar albino rats. It was also showed that the anti-diabetic activity of polyherbal formulation in suspension form is nearly comparable with standard drug glibenclamide (261.5 ±0.56) and the formulation at the dose of 200mg/kg was showed more significant antidiabetic effect at 1st hour(336.5± 0.76) after single dose drug administration than 400mg/kg body weight but the formulation at dose of 400mg/kg was also showed more significant effect at 4^{th} hours(304.16± 1.01) when its compare with the standard group (302.33±0.88) on Wistar albino rats and continue this significant effect till 24^{th} hours. So it was concluded that the formulation have significant anti-diabetic activity and also stable till the 3 month which was observed by the stability testing by using different parameters used for evaluation of suspension.[13]

Anand Duraiswamy et al (2016) studied the effect of polyherbal formulation for its antidiabetic activity. The formulation showed the presence of major constituents such as steroids, cardiac glycosides, phenols, flavonoids, and saponins. It also had a high level of phenols (340 ± 2.5 mg/g), flavonoids (235.4 ± 8.3 mg/g), and vitamin C (470.8 ± 16.6 mg/g), and showed a half-maximal inhibitory concentration (IC50) value of 0.41 ± 0.03 mg/mL and 0.51 ± 0.01 mg/mL for amylase and glucosidase, respectively. The results showed that ADJ6 had a significant inhibitory activity on alpha-amylase and alpha-glucosidase; however, its inhibitory activity was less than that of acarbose. The plants that are formulated in ADJ6 possess potent antidiabetic activity. Thus, we found that ADJ6 is a potent lead for effective diabetic management; however, an evaluation of the formulation must be illustrated using an in vivo model.[14]

Manjari Mittal et al, (2014) were developing combinational herbal oral antidiabetic suspension containing indigenous ethnomedicinal plants extract. The amount of individual plant's extract required for formulation was based on the effective dose of plant's extract. The maximum therapeutic effective dose for diabetic Wistar rats was found to be 50 mg/kg b.w. of Phyllanthus emblica, 50 mg/kg b.w. of Annona squamosa, 100 mg/kg b.w. of Curcuma longa and 100 mg/kg b.w. Berberis aristata. Suspension was prepared by using effective doses with suitable suspending and stabilizing agents. The formulated suspension was evaluated viz., organoleptic and accelerated stability studies at variable (different) temperature. Suspension exhibited pleasant appearance and texture; there were no changes in sedimentation, flow rate, pH, viscosity and other physiochemical parameters. All the stability parameters in formulated suspension are stable and acceptable. It is concluded that suspension of ethanolic extract of Phyllanthus emblica, Annona squamosa, Berberis aristata and methanolic extract of Curcuma longa formulated in combinational therapy could be effective and safe for antidiabetic use.[15]

Adedayo O. Ademiluyi, et al, (2015) in their study they investigated the phenolic constituents, antioxidant properties and effect of aqueous extracts from some Corchorus species (C. aestuans, C. bougoudo, C. capsularis, C. olitorius and C. urtifolicus) on alpha-amylase and alpha-glucosidase activities in vitro. The predominant phenolics detected by GCFID were kaempferol, rutin, apigenin, luteolin, Caffeic acid and quercetin. The results showed that all the Corchorus species significantly ($P < 0.05$) inhibited aamylase and a-glucosidase activities dose-dependently (0-8 µg/ml). C. olitorius had the highest alpha-amylase inhibitory ability while C. bougoudo demonstrated the maximum alpha -glucosidase inhibition. However, all the Corchorus species inhibited a-glucosidase better than alpha amylase and also exhibited antioxidant properties. The enzyme inhibitory and antioxidant properties exhibited by the Corchorus species could be attributed to their phenolic constituents and vitamin C content; a possible mechanism by which Corchorus species could elicit therapeutic effects on type 2 diabetes mellitus as obtained in folklore medicine.[16]

Bhise SB et al, (2009) studied the clinical efficacy of antidiabetic polyherbal Ayurvedic formulation Madhujeevan Churna containing Stevia rebaudiana on 45 NIDDM patients. Madhujeevan Churna was formulated using known Ayurvedic antidiabetic herbs- Curcuma longa, Aegle marmelos, Gymnema sylvestre, Azadirachta indica, Emblica officinalis, Salacia reticulata, Syzygium janbolanum, Momordica charantia and Stevia rebaudiana. Mild to moderate diabetics, whose glucose lowering effect is unsatisfactory, despite the use of conventional oral hypoglycemic were selected. The blood glucose levels were estimated by reported methods initially and then after 15 days on OPD basis up to 90 days. Clinical

examination, including patient's compliance and acceptability of the formulation was also evaluated. Madhujeevan Churna has shown marked glucose lowering effect. Stevia rebaudiana has enhanced the antidiabetic activity of other drugs, and masked their bitter taste. The result reveals that Madhujeevan Churna can be a safe, acceptable and effective alternative or adjuvant to the conventional oral hypoglycemic.[17]

Kumeshini Sukalingam et al, (2015) studies, they prepared formulation containing five medicinal plants (Mango, Guava, Amla, Garlic and Onion) with proven antidiabetic and related beneficial effects were selected for the preparation. The efficacy of prepared formulations were tested on Type II diabetic patients and compared with placebo diabetic control patients. The polyherbal formulations produced a significant decrease in blood glucose level after 8 weeks of treatment. Diabetic patients exhibited increase levels of lipid profiles such as cholesterol, triglycerides, and low density lipoprotein cholesterol (LDL-cholesterol), and a decrease in the level of high density lipoprotein cholesterol (HDL-cholesterol) during the first week of treatment. The elevated lipid profiles were restored to near normal by the treatment of polyherbal formulations for all the estimated parameters at 8^{th} week. In addition the polyherbal formulations also restored the BMI, blood pressure, haemoglobin, glycosylated haemoglobin and urinary parameters levels which indicates that they reduce the other complicacies of diabetes. The results of the formulations on Type II diabetic group were strong evidence that not only it restored the glycemic level but also reduced the lipid profile to the near normal. Based on the findings, the polyherbal formulations have significant antidiabetic potential for Type II diabetic patients.[18]

Sunil Mistry et al, (2012) studies evaluate the Hepatoprotective activity of polyherbal formulation (PHF) against carbon tetra chloride (CCl4) induced hepatotoxicity in rats, by subcutaneous administration of carbon tetra chloride (CCl4) with olive oil as a diluent in 1:1%v/v on 2nd and 3rd day. The liver damage was confirmed by estimation of elevated levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP) and serum bilirubin. Polyherbal formulation (PHF) pretreatment (300mg/kg/b.w.) significantly reduces the CCl4 induced elevated serum levels of SGOT, SGPT, ALP and serum bilirubin.[19]

Sakthivel E et al (2013) studies evaluates the pharmacological efficacy of the polyherbal formulation against benign prostatic hyperplasia using in vitro and in vivo studies. The pharmacological investigation both in vitro (evaluation using NIH 3T3 cell lines by MTT assay) and in vivo (activity against testosterone induced experimental hyperplasia in male

Wistar rats) were performed to evaluate the efficacy of the polyherbal formulation. From the in vitro study, IC50 of the herbal formulation was found to be 211.8µg/ml and the study showed the better inhibition of cellular proliferation of stromal cells. The study of antibacterial activity showed a good zone of inhibition against E.coli. Data obtained from the in vivo study suggests that the formulation has good efficacy against the prostatic hyperplasia in male Wistar rats on concentration dependent manner. The polyherbal formulation may be evaluated in clinical trials against benign prostatic hyperplasia.[20]

Sameena Alam et al, (2013) studies investigate the polyherbal formulation (Kalonji sugar powder) for antidiabetic effect in streptozotocin induced diabetes in Wistar rats. The polyherbal formulation (Kalonji sugar powder) was also investigated for its effect on serum lipid profile. Two doses of the water extract were chosen viz. 250 mg/kg b.w and 500 mg/kg b.w in Wistar rats. The aqueous extract at a dose of 500 mg/kg b.w showed significant ($p<0.01$) reduction in fasting blood sugar level to 61.3% after 14 days treatment. A preliminary phytochemical analysis revealed the presence of various phytoconstituents which were believed to be responsible for pharmacological activity. It brought about fall in the level of total cholesterol by 21% with increase of 45% in high density lipoprotein (HDL) cholesterol including 53.6% decrease in triglyceride level (TG) as compared to diabetic control animals. The present study reveals the fact that polyherbal formulation (Kalonji sugar powder) has antidiabetic effect. It reverses the abnormal lipid profile observed in diabetic animals. Therefore the water extract of this formulation is useful in maintaining healthy glucose levels and cholesterol levels.[21]

ENCLOSURE-III

6.3 NEED FOR THE STUDY

Circadian misalignment is associated with increased risk for obesity, diabetes, cancer, cardiovascular disease and many more chronic diseases. The changes in Circadian cycle and life style causes the imbalance in autonomic nervous and endocrine system function, this results a high blood pressure and impaired glucose uptake by the muscle on one hand and a high insulin secretion, increase in intra-abdominal and fatty liver on the other. An increasing number of epidemiological studies have reported an association between short sleep duration and higher risk of developing obesity and type II diabetes[22, 23.]

Melatonin, which occurs ubiquitously in nature, is a remarkable molecule with diverse physiological functions. Melatonin is involved in the seasonal control of reproduction, sleep regulation, immune mechanisms and regulation of circadian rhythmicity. It also have antioxidant and anti-inflammatory effects, melatonin also inhibits tumor growth and genetic mutation. As the prototype of the chronobiotic class of drugs, melatonin restores the phase and amplitude of circadian rhythmicity by interaction with MT1 and MT2 melatonin receptors expressed in the hypothalamic SCN and other brain regions. A number of studies indicate that melatonin has the ability to reduce obesity, type 2 diabetes and liver steatosis. Melatonin treatment induces regeneration/proliferation of β-cells in pancreas which leads to a decrement in blood glucose in streptozotocin induced type1 diabetic rats[22, 24.]

During digestion, certain foods can stimulate the production and / or stimulation of certain brain chemicals including melatonin. Furthermore, studies show that melatonin exhibits many bioactivities, such as antioxidant activity, anti-inflammatory characteristics, boosting immunity, anticancer activity, cardiovascular protection, anti-diabetic, anti-obese, neuroprotective and anti-aging activity. This review summaries the dietary sources and bioactivities of melatonin, with special attention paid to the mechanisms of action. Meanwhile, some foods with extremely high content of melatonin are of great value to be developed into functional foods, which would contribute to the prevention and treatment of various diseases[25]. Tryptophan, the precursor of melatonin and also of the neurotransmitter serotonin, is known to increase brain serotonin and melatonin levels during light and dark, respectively as well as the delta power and the amount of non-rapid eye movement sleep[26].

The philosophy behind *Ayurveda* is preventing unnecessary suffering and living a long healthy life. Unlike the allopathic medicines which uses mainly synthetic chemicals designed

for specific target receptors and primarily give symptomatic relief, *Ayurveda* involves the use of natural means such as diet, herbs, spices, minerals, exercise, meditation, yoga, mental hygiene, sounds, smells and mechano-procedures to eliminate the root cause of the disease by restoring balance, at the same time create a healthy life-style to prevent the reoccurrence of imbalance. *Ayurveda* is said to be holistic as it aims to integrate and balance body, mind and spirit to prevent illness and promote wellness, longevity, vitality and happiness, hence in the present study we are planned to prepare and assess the effectiveness of tryptophan and melatonin containing bioactive food compounds for management of chronic disorders in experimental animal.

ENCLOSURE -IV

6.4 AIM AND OBJECTIVES:

HYPOTHESIS: The tryptophan and melatonin containing bioactive food compounds plays an important role in management of chronic disorders through reducing hyperglycemia, plasma cholesterol and possessing antioxidant activity.

OBJECTIVES:

- The main object of the invention is to provide a safe and effective herbal formulation and a process for preparing the same, which prevents and treats diabetes and associated complications of diabetes.
- Another object of the invention is to provide a safe and effective herbal formulation and a process for preparing the same, which prevent the glycosylation of Hemoglobin. Glycosylated Hemoglobin causes long-term irreversible damages to eyes, kidneys, nerves and blood vessels.
- Yet another object of the present invention is to provide a safe and effective herbal formulation and a process for preparing the same, which improves the renal function and health of the diabetics, reduces the elevated levels of serum creatinine, decreases the incidence of urinary tract infections and improves nephropathy.
- Another object of the invention also relates to an herbal formulation for prevention and treatment of oxidative stress. Oxidative stress which results in numerous diseases and such as cancer, cardiovascular disease, neural disorders, Alzheimer's disease mild congnitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, ageing, atherosclerosis through are cytotoxic effect and tissue injuries.

ENCLOSURE-V

7 MATERIALS AND METHODS:

7.1 Source of data: Source and Collection of data:

The data is generated using laboratory experimental techniques. Albino rats and mice will be used for this purpose. Data will be acquired from national and international journals and also from other reputed libraries. The day to day development will be updated by conducting literature survey through e-publishing, journal, magazine and online sources.

Animals:

Albino mice 18-24g and albino rats weighing 150-250g will be used. The animals will be maintained under controlled conditions of temperature (23 ± 2^{o} C) and 12-h light-dark cycles. The animals will be randomized into experimental and control groups and housed each in two sanitized polypropylene cages containing sterile paddy husk as bedding. They will have free access to standard pellets as basal diet and water *ad libitum.*

ENCLOSURE-VI

7.2 Method of collection of data:

Phase 1: Collection and authentication of bioactive food compounds: The bioactive food compounds will be collected, authenticated, cleaned, dried and powdered for further process.

Phase 2: Preparation of tryptophan and melatonin containing Polyherbal Formulation

Phase 3: Preliminary phytochemical investigation: Preliminary phytochemical investigation of formulation is carried out by qualitative chemical test and chromatographic technique.

Phase 4: Determination of LD_{50}: The acute toxicity of formulations will be determined by using albino mice of either sex (20-25 g), maintained under standard conditions by applying QbD approach.

Phase 6: To investigate the effect of selected Polyherbal Formulation against oxidative stress

Phase 7: To evaluate the *in-vivo* and *in-vitro* anti-diabetic activity of the selected formulation in a translational animal model.

Data and statistical analysis: Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons between all groups were performed by using two tailed one-way ANOVA followed by the Dunnet 't' test. P-values <0.05 were considered statistically significant.

PLAN OF WORK

a. Literature survey

b. Collection and authentication of bioactive food compounds

c. Development and Preliminary phytochemical evaluation of tryptophan and melatonin containing Polyherbal formulation

d. Pharmacological evaluations of Polyherbal formulation for

- Acute Toxicity Study.
- **Antioxidant Activity[27]:**
- **Evaluation of antidiabetic activity**

1. **Hypoglycemic study in non-diabetic rats[28, 29]:** The blood glucose level of all groups rat was determined by GOD-POD kit following the given instruction by manufacturer.
2. **Antidiabetic study:**

   Nicotinamide-Streptozotocin (NAD-STZ) induced diabetic model[28, 30, 31]

   - ✓ Estimation of blood glucose level and creatinine
   - ✓ Estimation of biological parameters viz., hexokinase, glucose-6-phosphatase, fructose-16-bisphosphatase; lipid parameters viz., total cholesterol (TC), total triglyceride (TG), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL) and very low density lipoprotein (VLDL) etc.
3. ***In-Vitro* Evaluation of Anti-Diabetic Activity**
   a. Pancreatic lipase inhibitory activity[32]
4. **Effect of formulation on Haemoglobin Glycosylation[33]:** assess the glycosylation because Diabetes complications are primarily caused by 2 factors: Excessive Glycosylation and Sorbitol Accumulation.

e. Statistical interpretation

DURATION OF WORK:

S. No	Academic Year	Activity	Tentative Duration (months)
1	First year	• Literature Review	06
		• Collection and authentication bioactive food compounds and preparation of tryptophan and melatonin containing polyherbal formulation	06
2	Second year	• Phytochemical analysis and conduct *in-vivo* acute toxicity studies of polyherbal formulation	04
		• Assessment of *in-vitro* Antioxidant of polyherbal formulation by using experimental animal's model	04
		• Assessment of *in-vivo* Anti-Diabetic Activity of polyherbal formulation using experimental animal's model.	04
3	Third year	• Assessment of *in-vitro* Anti-Diabetic Activity of polyherbal formulation using experimental animal's model.	04
		• Compilation, interpretation of data and writing of thesis	08
Total			36

ENCLOSURE –VII

LIST OF REFERENCES:

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Research Student
Mr. Shivakumar S. Ladde
M. Pharm. (Pharmacology)
Centre: School of Pharmacy,
S.R.T.M.U. Nanded.

Research Guide
Dr. Bhusnure O. G. **M. Pharm, Ph. D.**
Principal
Channabasweshwar Pharmacy College (Degree),
Latur, Maharashtra, India - 413512